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► To cite this version:

Nicolas Cimetiere, Isabelle Soutrel, Marguerite Lemasle, Alain Laplanche, André Crocq. Standard addition method for the determination of pharmaceutical residues in drinking water by SPE-LC-MS/MS. Environmental Technology, 2013, pp.Published online. 10.1080/09593330.2013.800563 . hal-00870208

HAL Id: hal-00870208

<https://hal.science/hal-00870208>

Submitted on 6 Oct 2013

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1 **STANDARD ADDITION METHOD FOR THE DETERMINATION OF**
2 **PHARMACEUTICAL RESIDUES IN DRINKING WATER BY SPE-**
3 **LC-MS/MS**

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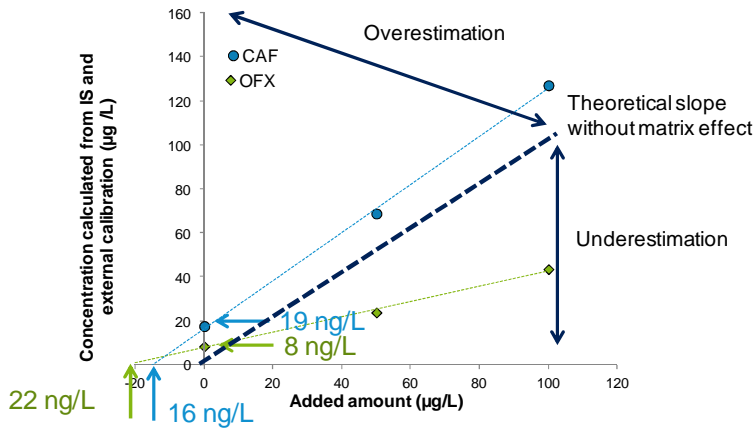
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Graphical Abstract



Abstract

The study of the occurrence and fate of pharmaceutical compounds in drinking or waste water processes has become very popular in recent years. LC-MS/MS is a powerful analytical tool often used to determine pharmaceutical residues at trace level in water. However, many steps may disrupt the analytical procedure and bias the results. A list of 27 environmentally relevant molecules, including various therapeutic classes and (cardiovascular, veterinary and human antibiotics, neuroleptics, non-steroidal anti-inflammatory drugs, hormones and other miscellaneous pharmaceutical compounds) was selected. In this work, a method was developed using Ultra Performance Liquid Chromatography coupled to tandem Mass Spectrometry (UPLC-MS/MS) and solid phase extraction (SPE) to determine the concentration of the 27 targeted pharmaceutical compounds at the nanogram per liter level. The matrix effect was evaluated from water sampled at different treatment stages. Conventional methods with external calibration and internal standard correction were compared to the standard addition method. An accurate determination of pharmaceutical compounds in drinking water was obtained by the

standard addition method associated with UPLC-MS/MS. The developed method was used to evaluate the occurrence and fate of pharmaceutical compounds in some drinking water treatment plants (DWTPs) in the west of France.

Key words: Pharmaceutical compounds; Multiresidue analysis; Ultra Pressure Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS); drinking water; standard addition method.

1. Introduction

Human and veterinary uses of pharmaceutical compounds lead to the releasing of bioactive compounds into the aquatic environment. Metabolization rates depend on the nature of the drugs and may range from 1 – 96 % [1]. Non-metabolized drugs are thus excreted in urine as free or conjugated forms [2,3], and collect in the waste water network. Pharmaceutical compounds are not completely removed during waste water treatment [4-7]. The efficiency of the process depends on the operating conditions and the nature of the molecule [6]. For example, conventional treatment with activated sludge effectively eliminates ibuprofen, and benzafibrate while diclofenac, carbamazepine and sulfamethoxazole are scarcely removed [8]. Some pilot scale studies have been carried out using a membrane bioreactor in an attempt to improve the outcome; these processes were found to be more efficient than activated sludge reactors at removing pharmaceutical compounds [8,9]. The disinfection of treated waste water can also improve the elimination rate of pharmaceutical compounds [10]. An indirect way of introducing these compounds into the environment is via agricultural activities. Sludge from waste water treatment plants may be spread on fields as

a fertilizer and the pharmaceutical compounds which can be immobilized in this sludge may then contaminate the soil [11]. The veterinary use of drugs can lead to a direct environmental contamination by the discharge of untreated effluent from intensive animal farming. Direct soil contamination can occur by the excretion of urine and feces by farm animals onto fields [12]. Rainfall and soil leaching may then transport pharmaceutical compounds from the soil to the aquatic compartment [13]. Intensive livestock farming is one of the main economic sectors of Brittany area (north-west France). Moreover, a large proportion of the population uses a non-collective waste water treatment to clean household effluent, so drinking water treatment specialists are beginning to be concerned about the potential contamination of water resources by pharmaceutical compounds. Some recent study shows that

Recently the French Agency for Food Health Safety (AFSSA) determined, the main relevant molecules to examine in drinking water from the total amount consumed and their properties in the aqueous phase [14]. Based on this work, a wide measurement campaign was carried out by the French Agency for Environmental and Occupational Health Safety (ANSES) [15]. From the 150 molecules included in the ANSES study, only 20 were found at concentrations above the the limit of quantification (LOQ) and 11 between the limit of detection (LOD) and the LOQ. Only these molecules were selected for the present work. Accurate trace determination of emerging contaminants in the environment is an important analytical challenge. The first obstacle is associated with the gap between environmental concentrations and the quantification limits of analytical systems. Pharmaceutical compounds concentrations range from LOQ of 2000 ng L⁻¹ to LOQ of 200 ng L⁻¹ in surface water and drinking water, respectively [16-19] while the LOQ of conventional MS-MS

apparatus (without a pre-concentration step), are typically in the $\mu\text{g L}^{-1}$ range. Consequently, a concentration step is needed before analysis; solid phase extraction (SPE) is the method of choice for the determination of emerging contaminants in water [20-22]. The second obstacle, resulting from the use of SPE, is the concomitant extraction of interfering species and the target molecules. Polar organic pollutants are commonly determined by liquid chromatography with tandem mass spectrometry (LC-MS/MS). However, interfering species may affect the analytical procedure at different stages: i) some compounds may react with targeted molecules during the sampling and storage periods, ii) organic or inorganic solutes may affect the yield of SPE extraction, iii) natural organic matter may coelute with targeted compounds which leads to a signal disrupting with under/overestimation or false positive samples [23]. The study presented here deals with the development of the method including an evaluation of the matrix effect. Accurate determination of pharmaceutical compounds in drinking water was performed by the standard addition method associated with ultra pressure liquid chromatography and tandem mass spectrometry. The method was used to evaluate the occurrence and fate of pharmaceutical compounds in some drinking water treatment plants (DWTP) in the west of France.

2. Materials and methods

2.1. Stock solution and standard preparation.

Stock solutions of individual pharmaceutical compounds were prepared by diluting reagent-grade chemicals (Sigma Aldrich) in methanol (Fisher). Ultra pure water (UPW) was provided by an ElgaPureLab System (18.2 M Ω .cm). Chromatographic solvents (MeCN;

MeCN with 0.1 % formic acid) were purchased from JT Baker (LC-MS grade) and were used in association with UPW or UPW with 0.1 % formic acid. A standard mix solution (5 and 10 mg L⁻¹ in MeOH) was prepared from individual stock solutions including all the targeted molecules except amoxicillin, caffeine, oxazepam and internal standards. The solution was then divided into a series of vial and stored at -20°C in the dark. The vial containing the standard mix was placed at room temperature before use and the unused amount was discarded. The standard mix was used to prepare both injections standard for the external calibration and spiked solutions for the standard addition method. Amoxicillin, caffeine, and oxazepam stock solution were prepared in UPW, MeOH and MeCN, respectively. Calibration curves were plotted using eight-level standard solutions (1, 2, 5, 10, 25, 50, 100, 200 µg L⁻¹ and up to 2000 µg L⁻¹ for caffeine). The chromatographic sequence consisted of the injection of standards and samples as follows: calibration curve - first samples analysis - calibration curve - second samples analysis - calibration curve. In addition a middle-range standard solution was injected every 10 injections in order to verify the absence of significant signal deviation.

2.2. Sample preparation

On arrival at the laboratory, water samples stored in 2-L amber glass bottles were filtered through a 0.45 µm cellulose acetate membrane to remove suspended matter and colloids. Samples were then stored in the dark before preparation and analysis. All the analyses were carried out within a maximum storage period of 5 days. Solid phase extraction was performed by filtering 200 mL of sample into a 6 mL Oasis HLB cartridge (6 cc, 150 mL, Waters). HLB cartridges were conditioned with 5 mL of MeCN and rinsed with 5 mL of UPW prior to the extraction step. Extraction was conducted by the filtering 200 mL of

sample (acidified at pH = 2 with sulfuric acid or not) under reduced pressure at a flow rate of approximately 3 mL min⁻¹. The cartridge was cleaned with 5 mL UPW or UPW acidified at pH = 2 (depending on the extraction method used) and then eluted with 4 mL MeCN. The extract was evaporated under nitrogen flow to obtain a final volume of 100 µL. 100 µL of internal standard (caffeine-¹³C₃ and ibuprofene-d₃, 100 µg L⁻¹ in MeCN/UPW 10/90) was added prior to LC-MS/MS analysis.

2.3. Liquid Chromatography Tandem Mass Spectrometry

All samples were analyzed using LC/MS/MS equipped with an electrospray ionization source (ESI). The analytical equipment consisted of an ultra pressure liquid chromatography system (Acquity, Waters) equipped with a reversed phase UPLC column from Waters (Acquity C18 BEH, 100 mm x 2.1 mm ID, 1.7µm) and thermostated at 45°C. The autosampler temperature was set at 4°C, and the injection volume was 5 µL in the full-loop mode. The mass spectrometer (Quattro Premier; Micromass) general operating conditions were : cone gas (N₂, 50 L h⁻¹, 120°C) -desolvation gas (N₂, 750 L h⁻¹, 350°C); collision gas (Ar, 0.1 mL min⁻¹); capillary voltage (3000 V). The advanced mass parameters (cone and collision cell voltage) are further described in Table 1.

3. Results and discussion

3.1. Optimization of mass spectrometry

Infusion is the first step of method development by liquid chromatography tandem mass spectrometry. It consists of a direct analysis of a pure diluted solution without separation in order to record the mass spectrum of each selected compound and to determine the MRM transitions. During this step the MS parameters such as cone voltage, and collision cell energy were optimized for each compound in order to achieve the maximum sensitivity.

Table 1 shows the results obtained for the 29 molecules studied here; 3 internal and recovery standards are also included. ESI is soft ionization technique which allows the selection of a pseudo-molecular ion as the parent ion for MRM transitions; ESI was used in both the negative and positive mode. The positive mode was selected for most of the molecules while 9 analytes were ionized under the negative mode. The pseudo-molecular ion ($[M+H]^+$ or $[M-H]^-$) was selected as the parent ion. When possible, simple fragment loss, such as water or carbon dioxide, was selected for the quantification or confirmation transition (parent ion \rightarrow daughter ion for the quantification and second daughter ion for confirmation). Only 1 transition was found for ibuprofen and ibuprofen- d_3 .

3.2. Chromatographic conditions and calibration

UPLC with a BEH C18 column was performed with a gradient of ultra-pure water / acetonitrile at $400 \mu\text{L min}^{-1}$. The effect of formic acid addition on the chromatographic separation was also evaluated. The starting eluent composition consisted of 19 % acetonitrile for 1 minute, which was then linearly increased to reach 95.5 % at 7.5 minutes. A final eluent containing 95.5 % acetonitrile for 2 minutes was used to clean the column and prevent any parasite peaks. In order to obtain an acceptable detection of all the molecules, 2 chromatographic conditions, with and without formic acid addition, to promote ionization, were needed (Figure 1). Separation was achieved in 6 minutes with a complete chromatographic run of 12 minutes. Caffeine- $^{13}\text{C}_3$ (CAF- $^{13}\text{C}_3$) and ibuprofen- d_3 (IBU- d_3) were used as the internal standard for quantification under the positive and negative ionization modes, respectively. Moreover, a recovery standard (ketoprofen- d_3) was added prior to the solid phase extraction; no correction relative to ketoprofen- d_3 was made

and its use was only indicative. External calibration curves were used for the determination of relative response factors (RRF) for each analyte according to the following equations:

$$\text{RRF (positive)} = \frac{\text{Analyte slope}}{\text{CAF} - {}^{13}\text{C}_3 \text{ slope}} \quad (\text{negative}) = \frac{\text{Analyte slope}}{\text{IBU} - \text{d}_3 \text{ slope}} \quad (\text{Eq. 1})$$

3.3. Linearity and quantification limits

Recovery rates (RR), linearity and quantification limits were determined at environmentally relevant concentrations; the results are summarized in Table 1. Because those obtained with UPW and surface water samples cannot be easily compared, the evaluation of the basic parameters of the validation method was carried out without organic interfering species (UPW or Evian water). Linearity was validated between 5 – 200 µg L⁻¹ in the vial (injected volume = 5 µL) which corresponds to 5 – 200 ng L⁻¹ in the starting sample if the RR is considered equal to 100 %. External calibration curves (8 levels + 1 blank) were also used to determine the standard deviation on the instrumental method; the SD presented here does not include the deviation on the SPE step. The results show that acceptable relative standard deviations lower than 10 % were obtained for most of the pharmaceutical compounds. However poor-quality results were obtained for hormones with a relative standard deviation ranging from 40 to 60 %.

The evaluation of instrumental detection (S/N = 3) and quantification limits (S/N = 10) (IDL and IQL) was performed by the injection of 10 blank samples (Evian water). From the 29 targeted compounds, IQL lower than 4 µg L⁻¹ were obtained for 27 of them, demonstrating that determination in the nanogram per liter range requires a concentration factor of up to 1000. Higher IQL values were obtained for ethinylestradiol and salicylic acid (8 and 24 µg L⁻¹, respectively). It should be underlined that the evaluation of the limit

of quantification (LOQ) by this method (apparatus LOQ without the SPE step and in the absence of interfering compounds) is not directly transposable for the determination of pharmaceutical residues in surface water. Nevertheless, this quick approach demonstrates that our method enables pharmaceuticals in surface and drinking water to be determined at an environmentally relevant concentration.

3.4. SPE extraction

Solid phase extractions were performed with Oasis HLB cartridges by filtering 200 mL of 0.45 μm pre-filtered sample in order to obtain a concentration factor of 1000. Because the selected molecules can be assumed to be weakly basic or weakly acid compounds, the effect of sample the acidification on the extraction yield was evaluated in UPW. Standard solutions each containing 100 ng L⁻¹ of analyte were filtered onto an HLB cartridge as previously described. Recovery rates were determined using the internal standards caffeine-¹³C₃ and ibuprofen-d₃ for the analysis under ESI+ and ESI-, respectively (Figure 2).

The results of the extraction experiments are summarized in Table 1. Acetaminophen, caffeine, carbamazepine, and oxazepam were almost quantitatively (80–120%) recovered in conditions all investigated. These analytes are assumed to be neutral drugs, which explains their high recovery yields under acidic and neutral extractions. In spite of a pK_a value of 4.16, a similar result was obtained for losartan. Amphoteric drugs such as danofloxacin and ofloxacin exhibited higher recovery yields under acidic extraction than under neutral conditions. Thus, for these compounds, the SPE is controlled by the carboxylic function and the amino group does not affect the extraction yield. The opposite effect was observed for amoxicillin where no acceptable recovery yields were obtained under acidic or neutral conditions. In this case, the controlling group should be the amino acid function and

extraction under basic conditions could increase the recovery yield. Extraction under acidic conditions was selected for most of the carboxylic acids, for example ibuprofen, ketoprofen and salicylic acid. In contrast to acidic drugs, basic drugs containing an amino group (i.e. atenolol, naftidrofuryl and lincomycin) had comparatively higher recoveries under neutral conditions due to the formation of ammonium derivatives at low pH values. Except for amoxicillin, the combination of both acidic and neutral extractions provided acceptable recovery rates for all the analytes. However the recovery rates determined in UPW experiments could be dramatically affected by the presence of interfering species (i.e. natural organic matter).

3.5. Evaluation of the matrix effect

The presence of organic or inorganic substances could lead to an analytical bias. Natural Organic Matter (NOM) is a complex mixture of polyfunctional macromolecules [24] which may disturb the SPE step, or MS ionization. From the various effects attributable to the presence of NOM some phenomena can be described such as competitive adsorption on the HLB phase [25], the formation of NOM-analyte complexes [26] and the modification of the analyte ionization efficiency in the MS source [27]. Although the presence of NOM is frequently associated with an underestimation of the targeted analytes (decreasing the extraction yield and/or the ionization efficiency), the opposite effect may also occur, despite not being well documented.

In order to evaluate the effect of NOM, the recovery rates obtained in pure water were compared with those obtained in surface water. Four surface waters (used to supply drinking water treatment plants) were spiked with stock solutions of pharmaceutical compounds to obtain a concentration of 100 ng L⁻¹ of each targeted analyte. Because

239 surface water may initially contain some pharmaceutical residues, unspiked samples were
240 also analyzed to determine the signal contribution due to the presence of analyte in surface
241 water; signal was then corrected to be specific to the added amount of analyte. Figure 3
242 shows the comparison between the recovery rates obtained in pure water and those obtained
243 in raw water (surface water) from the drinking water treatment plant A and B (DWTPA-
244 RW ; DWTPB-RW). These results demonstrate that the determination of pharmaceutical
245 compounds at trace level is very influenced by the water quality. For some compounds,
246 such as tylosin, atenolol, losartan, ibuprofen and amoxicillin, no significant matrix effect
247 was observed. The recovery rate determined for amoxicillin in surface water was quite
248 similar to that observed in pure water. However, due to its very low value, a possible matrix
249 effect may be masked. The absence of a detectable matrix effect on ibuprofen can be
250 explained by the fact that this compound was quantified relative to ibuprofen-d₃. Figure 3
251 shows a significant underestimation of diclofenac and β -estradiol in surface water. In
252 contrast, many compounds such as carbamazepine and epoxy-carbamazepine were
253 overestimated. The recovery rate observed for oxazepam in pure water (105 %) was not
254 significantly different from that observed in DWTPA-RW (104%) but a significant
255 overestimation was observed in DWTPB-RW (145 %). In the case of ethinylestradiol,
256 recovery rates in pure water and DWTPA-RW (108 and 89 %, respectively) were quite
257 similar whereas a significant underestimation was measured in DWTPB-RW (59 %).
258 Clearly, the recovery rates determined with pure water are not transposable to surface
259 water. The recovery rates obtained with surface water differ depending on the nature of the
260 NOM. Therefore, a classical approach with external calibration and internal/external

standard correction is not sufficiently accurate for the multi-residue analyses of pharmaceutical compounds at trace level in water.

3.6. Standard addition method

The standard addition method (SAM) is very efficient for correcting the matrix effect and providing an overall evaluation of this effect on both the SPE step and MS ionization. Moreover, it can be used even if the molecules were not initially present in water. All samples were spiked with stock solutions containing the 29 targeted pharmaceuticals (not spiked; 50 and 100 ng L⁻¹). The conventional quantification method (external calibration with internal standard correction) was compared with SAM results according to the following equations:

$$\text{conventional method: } [\text{Analyte}] = \frac{\text{Analyte Area}}{\text{IS Area}} \times \frac{[\text{IS}]}{\text{RRF}} \text{ with } [\text{IS}] = 100 \mu\text{g L}^{-1} \quad (\text{Eq. 2})$$

$$\text{SAM method: } [\text{Analyte}] = \frac{\text{Measured signal in unspiked sample}}{\text{slope of the standard addition calibration curve}} \quad (\text{Eq. 3})$$

Figure 4.a shows an example of matrix effect evaluation for some molecules not detected in the raw water of DWTP A. In the absence of a matrix effect, a theoretical slope equal to 1 should be obtained; in the present case (DWTP A-RW), some compounds such as estrone and sulfadimerazine were weakly affected by water quality and interfering species. Conversely, the low recovery rate obtained for diclofenac could be attributed to a decrease in the extraction yield and/or signal suppression caused by a modification of ionization in the ESI source. The inverse effect was observed for naftidrofuryl, for which conventional quantification leads to an overestimation. Because no signal attributable to naftidrofuryl was observed in the non-spiked sample, the overestimation could not be due to the co-

elution of a false-positive compound, but it could be caused by an ion enhancement effect. This type of matrix effect has previously been reported in the literature [23] with similar compounds (basic drugs) in surface water. Moreover, Dams et al. [28] underlined that ESI was especially susceptible compared to APCI. The same approach was adopted with compounds initially observed in the non-spiked sample (Figure 4.b). In the case of caffeine, similar results were obtained with the conventional method ($19 \pm 3 \text{ ng L}^{-1}$) and SAM ($16 \pm 3 \text{ ng L}^{-1}$). However, the quantification of ofloxacin by the conventional method ($8 \pm 2 \text{ ng L}^{-1}$) led to a significant underestimation (SAM: $22 \pm 3 \text{ ng L}^{-1}$) of its concentration in drinking water.

The standard addition method was used to determine the concentration of pharmaceuticals at different treatment stages from raw water to drinking water in four drinking water treatment plants (DWTPs). The matrix effect was evaluated on a total of 16 samples. The slopes of the curves, obtained with the 29 targeted compounds in the different samples (example given in Figure 4), are summarized as a box plot (Figure 5.). These results underline that the chromatographic method proposed here fails to determine the 29 targeted compounds accurately. Recovery rates obtained for amoxicillin were lower than 3 %, which could be explained by the extraction step (SPE yield lower than 7 % in pure water). Moreover, in some cases amoxicillin was not detected in the spiked samples (50 and 100 ng L^{-1}), so a competitive effect on the adsorption step and/or signal suppression could be suggested in addition to poor SPE efficiency. Not only was salicylic acid dramatically affected by the matrix effect, but antagonistic effects (signal suppression and enhancement) were also observed with similar water qualities: large signal suppression was observed in the raw water of DWTP A while signal enhancement occurred after the sand filtration step

of the same DWTP. A review of the chromatographic data also reveals an abnormally large area associated with salicylic acid. In some cases, the calculated concentrations with both the conventional and standard addition methods reach the milligram per liter range, so a cross-talk effect could be suggested. As smaller deviations between the conventional method and SAM were observed for compounds which were quantified relative to their analogous IS (ibuprofen, caffeine), the results obtained here demonstrate that the correction of the matrix effect with internal standards cannot easily be transposed to other compounds. In spite of the efficiency of the SAM to correct the matrix effect, amoxicillin and salicylic acid were removed from the quantifiable list of compounds; thus only the 27 of the 29 pharmaceutical compounds initially targeted were accurately quantified by the method proposed here.

3.7. Application to drinking water analysis

Concentrations of pharmaceutical compounds in the samples from DWTP were calculated from Equation 3. The results obtained during the sampling campaign show that only 13 molecules were observed at concentrations above the LOD at least once. Figure 6 summarizes the occurrence and fate of the detected compounds in the four sampled DWTPs. Concentrations observed ranged from the LOQ to 95 ng L⁻¹ (hydroxy-ibuprofen in DWTP D). From the 13 detected molecules, only 3 pharmaceutical compounds were quantified in all samples (caffeine, ofloxacin, hydroxy-ibuprofen). 9 molecules were detected with concentrations lower than the LOQ and 3 of these were only observed in raw water (losartan, epoxy-carbamazepine and ketoprofen). Erythromycin, tylosin, progesterone, hydrochlorothiazide and ibuprofen were detected (<LOQ) at different stage of the water treatment. Finally 6 targeted compounds were never detected during the

sampling campaign (lincomycin, diclofenac, estrone, pravastatin, atenolol and doxycycline). It should be underlined that the SAM approach identified significant signal inhibition of danofloxacin and ofloxacin in the raw water of DWTP B and in the chlorinated water of DWTP A. Since the spiking of danofloxacin and ofloxacin does not lead to a significant increase in peak area associated with these compounds, their quantification was not possible. Nevertheless, ofloxacin and danofloxacin were accurately determined after sand filtration at a concentration ranging from 5 – 10 ng L⁻¹, so it could be suggested that they were initially present in the raw water. This particular case reinforces the efficiency of the SAM approach for identifying matrix effects and facilitating the interpretation of results. Only the quantified compounds were considered when examining the effect of the water treatment process on the removal of pharmaceuticals (Figure 6). From the results obtained, 3 classes of pharmaceuticals can be defined. Several compounds, such as caffeine, trimethazine and oxazepam were partially removed during the treatment process. The clarification step (coagulation-flocculation-sand filtration) seemed to be most efficient for eliminating pharmaceutical compounds. In fact, acetaminophen, carbamazepine, amlodipine, sulfamethazine, β -estradiol and ethinylestradiol were completely removed after this step. These data are consistent with the work of Vieno et al. who demonstrated that coagulation of surface water with ferric sulfate could efficiently remove some pharmaceutical residues [29]. A second class of compounds can be defined as refractory pollutants; ofloxacin, danofloxacin and naftidrofuryl were not significantly eliminated during drinking water production. The third group of molecules consists of metabolites formed during water treatment; only hydroxy-ibuprofen in the present study. A large increase in hydroxy-ibuprofen concentration was observed in all the DWTPs

considered although ibuprofen was never observed at a concentration level above the LOQ. The glucuronide conjugate of ibuprofen is the main metabolite from ibuprofen metabolism [3]. Cleavage of this conjugate could occur during water treatment releasing the free form of ibuprofen, which could then be oxidized to produce hydroxy-ibuprofen. A similar mechanism has previously been proposed by Ternes et al. to explain the formation of estrone from the glucuronide conjugate of β -estradiol in a waste water treatment plant [[30]].

4. Conclusion

In this study, a multiresidue analysis of pharmaceuticals at trace level in surface and drinking water involving a solid phase extraction followed by UPLC-MS/MS determination was developed. Matrix effects were examined for 29 pharmaceuticals in 16 samples. Matrix effects were severe, even with internal standard correction, so the standard addition method was necessary for an accurate determination. The analytical method developed here was then used to evaluate the occurrence and fate of drug residues in drinking water treatment plants. Further studies will be conducted to confirm the effect of the water treatment process on the elimination of pharmaceutical residues.

366 REFERENCES

- 367 [1] M.S. de Graaff, N.M. Vieno, K. Kujawa-Roeleveld, G. Zeeman, H. Temmink,
368 C.J.N. Buisman, *Water Res.* 45 (2011) 375.
- 369 [2] P. Adler, T. Steger-Hartmann, W. Kalbfus, *Acta Hydrochim. Hydrobiol.* 29 (2001)
370 227.
- 371 [3] N.M. Davies, *Clin. Pharmacokinet.* 34 (1998) 101.
- 372 [4] M.L. Janex-Habibi, A. Bruchet, T.A. Ternes, *Tech., Sci., Methodes* 11 (2004) 59.
- 373 [5] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. von
374 Gunten, B. Wehrli, *Science* 313 (2006) 1072.
- 375 [6] C. Miège, J.M. Choubert, L. Ribeiro, M. Eusèbe, M. Coquery, *Environ. Pollut.* 157
376 (2009) 1721.
- 377 [7] T.A. Larsen, J. Lienert, A. Joss, H. Siegrist, *J. Biotechnol.* 113 (2004) 295.
- 378 [8] M. Clara, B. Strenn, O. Gans, E. Martinez, N. Kreuzinger, H. Kroiss, *Water Res.* 39
379 (2005) 4797.
- 380 [9] J. Radjenovic, M. Petrovic, D. Barceló, *Anal. Bioanal. Chem.* 387 (2007) 1365.
- 381 [10] G. Hey, A. Ledin, J.I.C. Jansen, H.R. Andersen, *Environmental Technology* 33
382 (2011) 1041.
- 383 [11] J. Radjenović, M. Petrović, D. Barceló, *Water Res.* 43 (2009) 831.
- 384 [12] I.S. Ruhoy, C.G. Daughton, *Environ. Int.* 34 (2008) 1157.
- 385 [13] A. Karnjanapiboonwong, J. Suski, A. Shah, Q. Cai, A. Morse, T. Anderson, *Water,*
386 *Air, Soil Pollut.* 216 (2011) 257.
- 387 [14] AFFSA, in, 2008.
- 388 [15] ANSES, in, 2011.
- 389 [16] H. Budzinski, A. Togola, *Environ. risques & santé* 5 (2006) 248.
- 390 [17] M. Farré, I. Ferrer, A. Ginebreda, M. Figueras, L. Olivella, L. Tirapu, M. Vilanova,
391 D. Barceló, *J. Chromatogr., A* 938 (2001) 187.
- 392 [18] P.E. Stackelberg, J. Gibbs, E.T. Furlong, M.T. Meyer, S.D. Zaugg, R.L. Lippincott,
393 *Sci. Total Environ.* 377 (2007) 255.
- 394 [19] O.A.H. Jones, N. Voulvoulis, J.N. Lester, *Environmental Technology* 22 (2001)
395 1383.
- 396 [20] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, *J. Chromatogr., A* 1161 (2007)
397 132.
- 398 [21] M. Gros, M. Petrović, D. Barceló, *Anal. Bioanal. Chem.* 386 (2006) 941.
- 399 [22] C. Stavrakakis, R. Colin, V. Hequet, C. Faur, P. Le Cloirec, *Environmental*
400 *Technology* 29 (2008) 279.
- 401 [23] J. Van De Steene, W. Lambert, *J. Am. Soc. Mass Spectrom.* 19 (2008) 713.
- 402 [24] E.M. Thurman, *Organic Geochemistry of Natural Waters*, Kluwer Academic,
403 Boston, MA, USA., 1985.
- 404 [25] F. Tamtam, F. Mercier, J. Eurin, M. Chevreuil, B. Le Bot, *Anal. Bioanal. Chem.*
405 393 (2009) 1709.
- 406 [26] H.-C. Holten Lützhøft, W.H.J. Vaes, A.P. Freidig, B. Halling-Sørensen, J.L.M.
407 Hermens, *Environ. Sci. Technol.* 34 (2000) 4989.
- 408 [27] R.J.C.A. Steen, A.C. Hogenboom, P.E.G. Leonards, R.A.L. Peerboom, W.P.
409 Cofino, U.A.T. Brinkman, *J. Chromatogr., A* 857 (1999) 157.

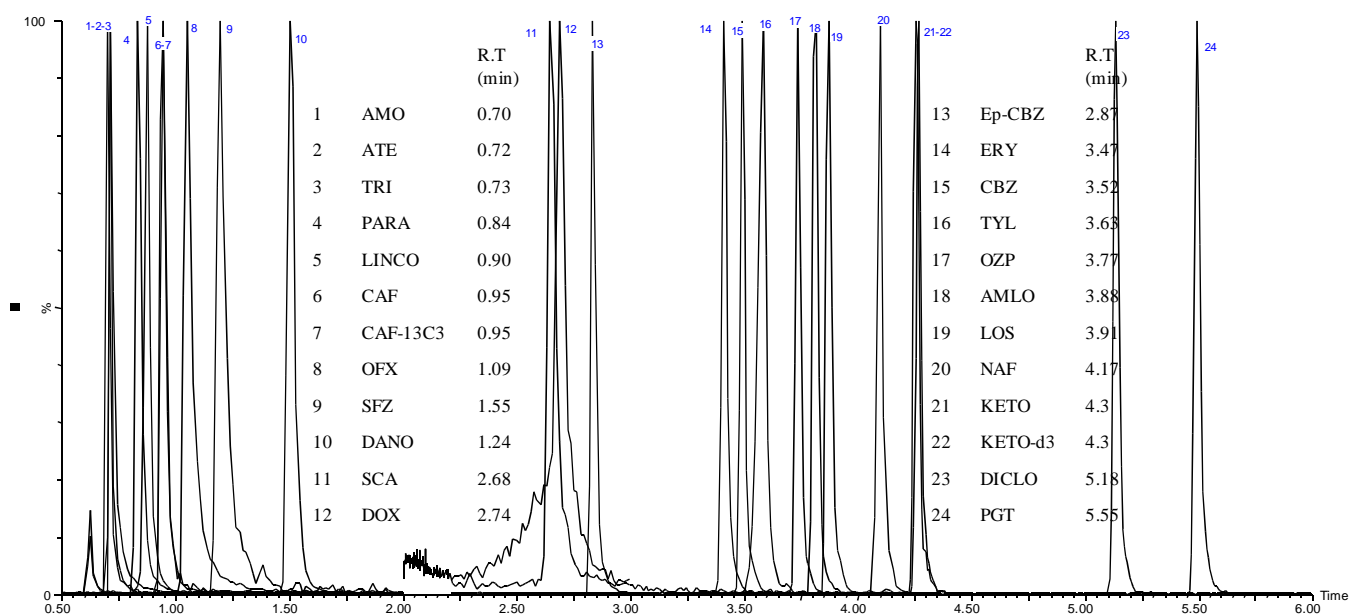
- 410 [28] R. Dams, M.A. Huestis, W.E. Lambert, C.M. Murphy, J. Am. Soc. Mass Spectrom.
411 14 (2003) 1290.
412 [29] N. Vieno, T. Tuhkanen, L. Kronberg, Environmental Technology 27 (2006) 183.
413 [30] T.A. Ternes, P. Kreckel, J. Mueller, Sci. Total Environ. 225 (1999) 91.
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Table 1. Summary of the method development

Therapeutic class	Molecule	MW (g/mol)	ESI (+ / -)	MRM transitions (m/z)			r ²	SD	LOD (µg L ⁻¹)	LOQ (µg L ⁻¹)	SPE	UPLC
				Parent ion. (CV ^a)	Quant. ion (CE ^b)	Conf. ion (CE)						
Cardiovascular	Amlodipine (AML)*	567.05*	+	409.6 (18)	238.1 (11)	292.2 (13)	0.994	0.109	0,3	1,0	^c A	A
	Atenolol (ATE)	266.34	+	267.0 (34)	145.0 (26)	74.0 (23)	0.986	0.118	0,2	1,0	^d N	A
	Losartan (LOS)*	461.00*	+	423.6 (30)	405.2 (12)	207.0 (22)	0.987	0.100	0,4	1,0	^e N+A	A
	Naftidrofuryl (NAF)*	473.56	+	384.6 (40)	99.7 (21)	84.7 (25)	0.992	0.106	1,4	2,0	N	A
	Pravastatin (PRA)*	446.51	-	423.2 (34)	100.6 (23)	321.1 (16)	0.988	0.119	0,1	1,0	N	N
	Trimetazidine (TRI)*	339.26	+	267.4 (21)	180.9 (16)	165.8 (26)	0.995	0.082	0,2	1,0	N	A
Antibiotics	Human	Amoxicillin (AMO)	+	366.5 (16)	113.7 (24)	349.0 (10)	0.980	0,176	0,4	1,0	A	A
		Doxycycline (DOX)*	+	445.5 (30)	428.2 (18)	153.8 (28)	0.986	0,103	0,7	2,0	A	A
		Erythromycin (ERY) *	+	734.2 (28)	158.0 (30)	576.2 (19)	0.987	0,123	0,0	1,0	N	A
		Ofloxacin (OFX)	+	362.0 (34)	318.0 (19)	261.0 (28)	0.985	0,155	0,9	2,0	A	A
	Veterinary	Danofloxacin (DANO)	+	358.5 (35)	314.0 (19)	283.0 (25)	0.976	0,210	1,8	4,0	A	A
		Lincomycin (LINCO)*	+	407.6 (40)	125.9 (28)	359.3 (18)	0.984	0,125	0,0	1,0	N	A
		Sulfadimerazine (SFZ)	+	279.4 (29)	185.9 (16)	91.7 (26)	0.979	0,133	0,3	1,0	N	A
		Tylosin (TYL) *	+	917.0 (60)	174.0 (37)	773.0 (29)	0.994	0,088	0,3	1,0	N	A
Neuro.	Carbamazepine (CBZ)	236.27	+	237.1 (28)	194.0 (19)	179.0 (39)	0.976	0.122	0,1	1,0	N+A	A
	Epoxycarbamazepine (Ep-CBZ)	252.27	+	253.3 (28)	179.9 (28)	236.0 (12)	0.988	0.111	0,3	1,0	A	A
	Oxazepam (OZP)	286.71	+	287.4 (34)	241.0 (20)	269.1 (14)	0.985	0.109	0,5	2,0	N+A	A
NSAID	Diclofenac (DICLO)	294,14	+	296,1 (22)	250,0 (10)	214,1 (25)	0.987	0.120	0,2	1,0	N	A
	Ibuprofen (IBU)	206,28	-	205,0 (17)	161,0 (7)	/	0.965	0.188	0,2	1,0	A	N
	Hydroxyibuprofen (OH-IBU)	222,28	-	221,2 (19)	177,0 (9)	158,7 (13)	0.994	0.103	0,8	2,0	A	N
	Ketoprofen (KETO)	254,28	+	255,0 (29)	209,0 (12)	105,0 (22)	0.989	0.085	0,3	1,0	A	A
	Salicylic acid (SCA)	138,12	-	137,0 (30)	92,6 (14)	64,7 (28)	0.984	0.100	9,1	24,0	A	A
Misc.	Acetaminophen (PARA)	151,16	+	152,0 (25)	110,0 (15)	90,0 (10)	0.986	0.094	1,3	4,0	N+A	A
	Caffeine (CAF)	194,19	+	195,1 (37)	137,7 (18)	109,7 (22)	0.987	0.129	1,8	3,0	N+A	A
	Hydrochlorothiazide (HCTZ)	297,74	-	296,2 (42)	77,6 (28)	204,8 (22)	0.981	0.170	0,1	1,0	A	N
Hormones	Ethinylestradiol (EE)	296,40	-	295,2 (54)	144,9 (40)	183,0 (35)	0.873	0.406	2,8	8,0	A	N
	17β-Estradiol (βE)	272,38	-	271,1 (50)	145,0 (38)	183,0 (41)	0.741	0.611	0,4	1,0	A	N
	Estrone (EO)	270,37	-	269,1 (53)	145,0 (35)	183,0 (36)	0.875	0.393	0,5	1,0	A	N
	Progesterone (PGT)	314,46	+	315,2 (32)	97,0 (24)	109,0 (26)	0.992	0.097	0,2	1,0	N	A
IS	Ketoprofen-d3 (KETO-d3)	257,30	+	258,4 (25)	212,0 (15)	179,8 (23)					N+A	A
	Caffeine- ¹³ C ₃ (CAF-13C3)	195,19	+	198,2 (35)	139,7 (20)	111,7 (22)					/	A
	Ibuprofene-d ₃ (IBU-d3)	209,30	-	208,2 (18)	163,9 (7)	/					/	N

^aCone Voltage in volt; ^bCollision energy in volt; ^cSPE extraction at pH = 2; ^dSPE extraction at pH = 7; ^eMean value of the 2 methods. *molecule whose molecular weight of the commercial product purchased does not correspond to the molecular weight of the active compound (i.e. amlodipine besylate – MW = 567.05 versus amlodipine – MW = 408.87)

a)



b)

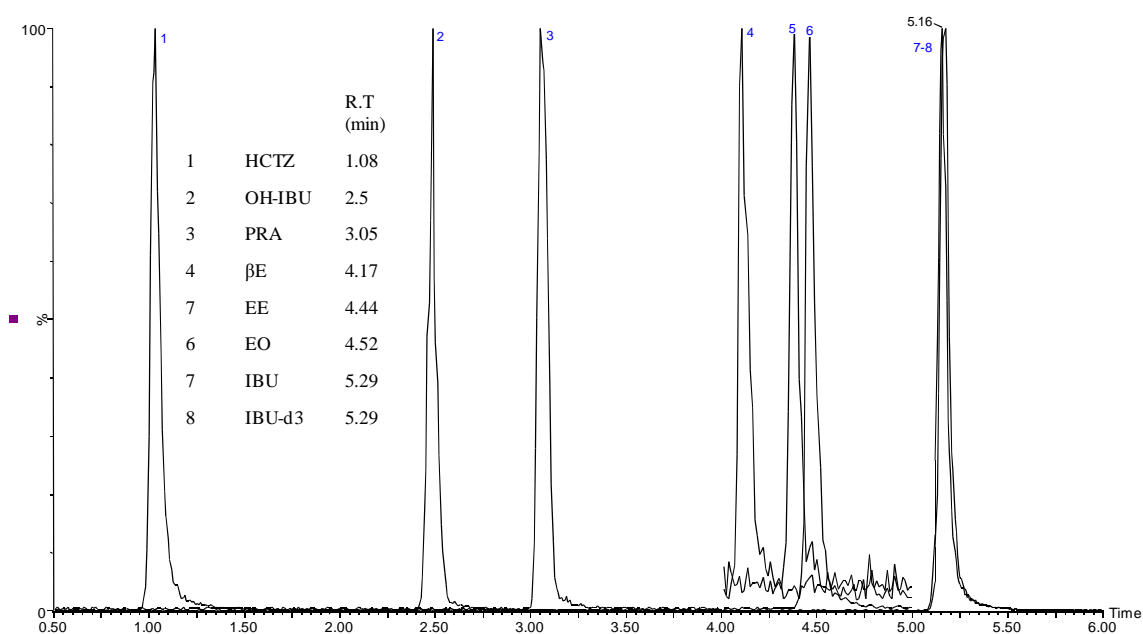


Figure 1. Example of chromatogram obtained with a standard mix solution at $100 \mu\text{g L}^{-1}$. Chromatograms obtained with (a) addition of 0.1% formic acid and (b) without acidification.

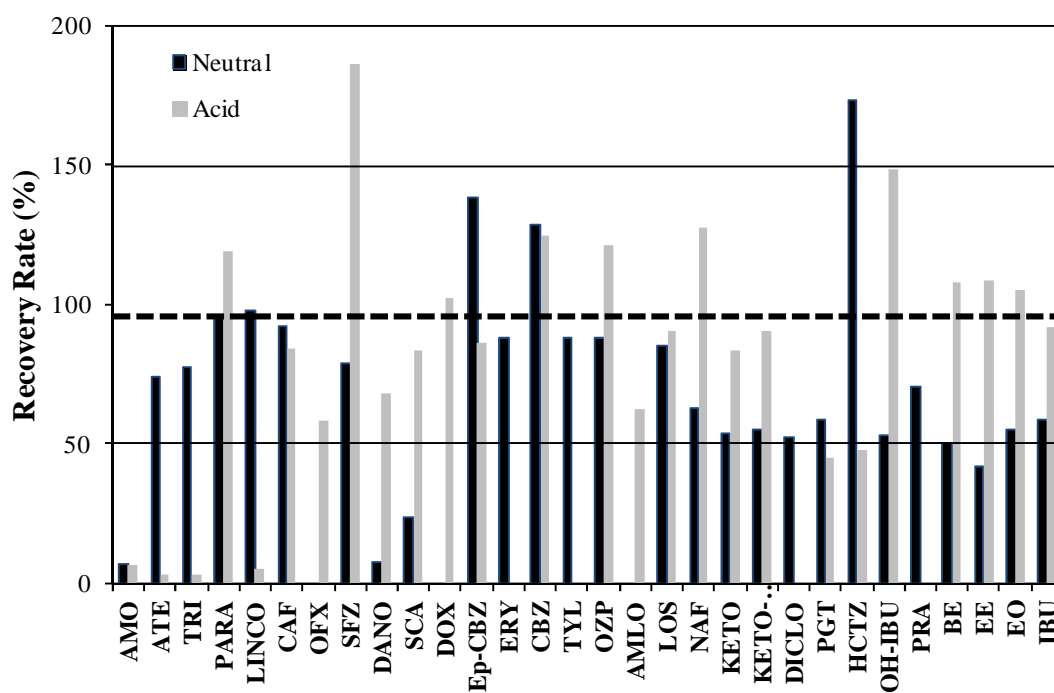


Figure 2. Effect of pH during SPE extraction on the recovery rate in pure water.

[Analyte] = 100 ng L⁻¹ ; concentration factor = 1000.

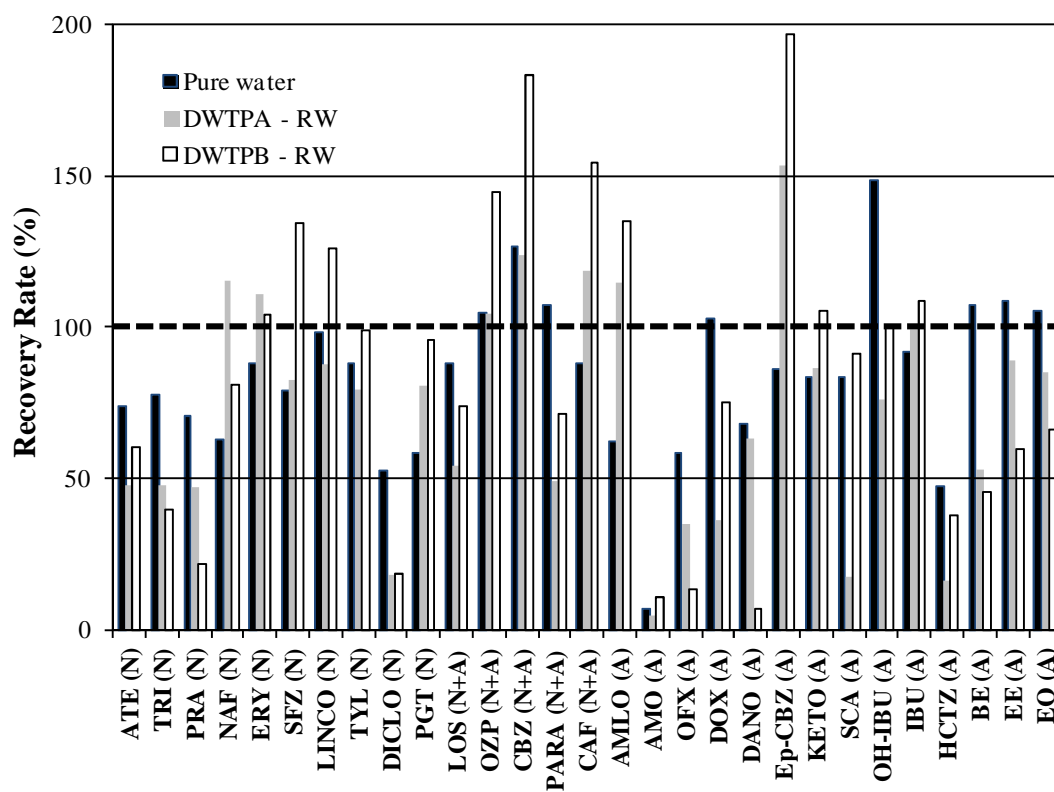
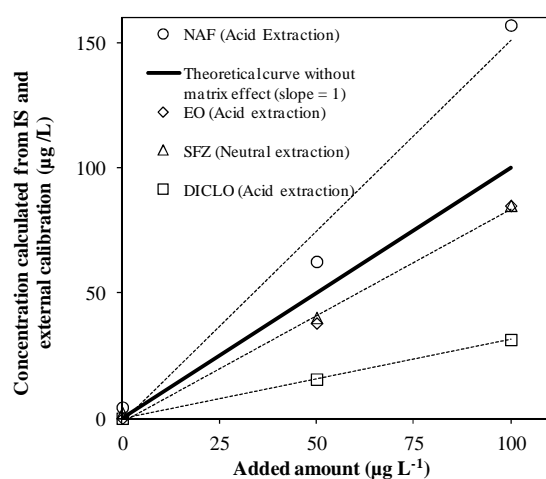


Figure 3. Effect of water quality on the recovery rate. [Analyte] = 100 ng L⁻¹ ; concentration factor = 1000 ; letters in parentheses refer to the SPE mode i.e. Acid and/or Neutral conditions.

a/



b/

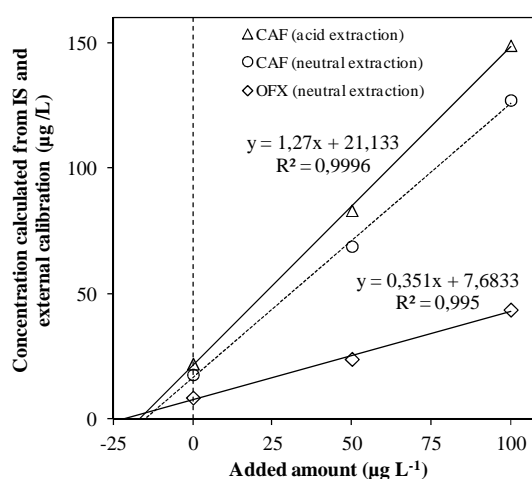


Figure 4. Comparison of concentrations determined by external calibration with internal standard correction and standard addition method. Example of compounds (a) not detected and (b) detected in DWTP A-RW.

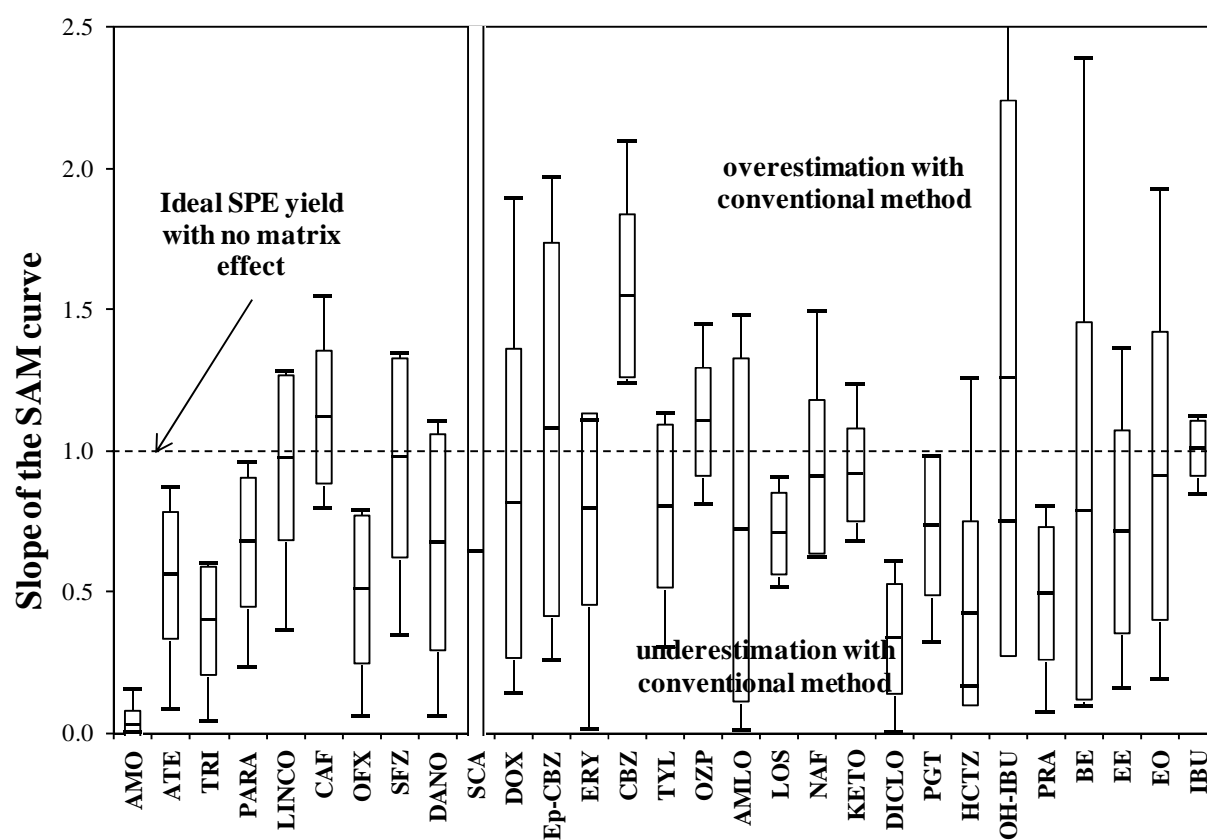


Figure 5. Overall evaluation of the matrix effect in multiresidue analysis of pharmaceutical compounds in surface and drinking water.

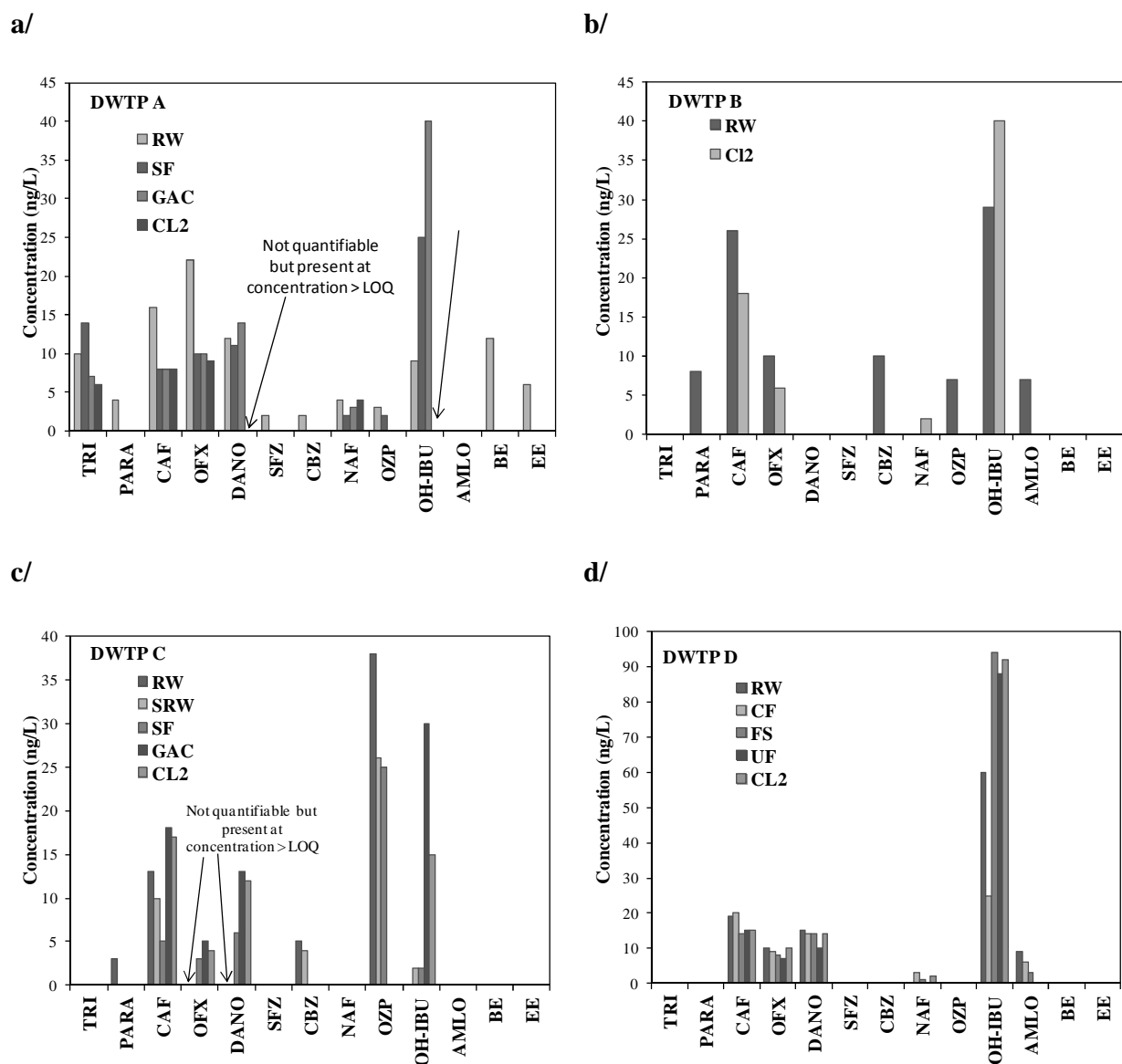


Figure 6. Fate and occurrence of pharmaceutical compounds at different stages of the drinking water process. Raw Water (RW), Stored Raw Water (SRW), Coagulation-Flocculation (CF), Sand Filtration (SF), Granular Activated Carbon (GAC) and Chlorination (CL2). DWTP A and B include an ozonation step before GAC filtration; DWTP B and D include a powder activated carbon reactor in the clarification step and a membrane ultra-filtration (UF) as a polishing treatment before chlorination.